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Florida Volatile Oils

IV. Sweet Orange*

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During the last decade the production of canned citrus juices in the state of Florida has rapidly increased. This has been brought about by several factors involving supply and demand. An over-supply of citrus fruits has brought about every possible utilization of them including their by-products. Research in this field has brought about excellent canning methods and products. Demand for the same has been increased by quantity production, lower prices, advertising and the desire on the part of the public to use these juices for their vitamin content and as a component in mixed drinks.

The result of the above situation has led to the development and sale of many citrus by-products which absorb to no small extent the cost of production. Among these by-products the essential oils, particularly the oil of sweet orange, have now come to be very important. Inasmuch as this is a relatively new development in Florida, much more scientific information is needed about these oils and particularly the oil of sweet orange which is produced in larger volume than the others. For this reason this investigation was entered upon.

The following materials were used:

1. Seventeen pounds oil of sweet orange, supplied by Essential Oil Producers Inc., Dunedin, Fla. This oil was obtained by them as a general run of oil in the 1940–1941 season and had been stored in their tanks for 3 months. It was used for most of this work.

2. One pound of oil of sweet orange supplied by Dr. Phillips Canning Co., Orlando, Fla. This oil was expressed by them in 1939–1940 and was used only for ester determinations.

3. Two ounces oil of sweet orange supplied by Florida Fruit Canners Co., Frostproof, Fla. This oil was expressed by them in 1939–1940 and was only used for ester determinations and flavoring properties.

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It is realized that these oils will vary in composition because of many factors such as variety, soil and growing conditions and processing. That oils vary from year to year is well known. There is no such thing as a true Florida Oil of Sweet Orange, because different lots will vary due to these conditions. If the Florida producers would unite in the blending and marketing of their oils in an effort to maintain the same quality from year to year, then we would have a truly "Florida Oil of Sweet Orange."

Because of the short period of this investigation, it was necessary to rely on single lots supplied by different producers. All oil was machine expressed.

The purpose of this investigation was to determine the physical, chemical and flavoring properties of the oil as well as to find a suitable method for making concentrates and determining wax content.

EXPERIMENTAL

Physical Constants.—The oil met all the U. S. P. XI physical standards except for the index of refraction of the 10% distillate. Found: deep orange color, cloudy solution with 2 volumes of 90% alcohol, soluble in all proportions in dehydrated alcohol, in carbon disulfide and in an equal volume of glacial acetic acid. Sp. gr.$^{25}_{25}$ 0.8425; $[\alpha]^{25}_{D}$ +95.5°; n^{20}_{D} 1.4734. U. S. P. XI acid, heavy metals and residue tests O.K. 10% distillate $[\alpha]^{25}_{D}$+97.5°, n^{20}_{D} 1.4729, difference of refractive index with original (1.4734 − 1.4729) 0.0005, required difference 0.0008 to 0.0015.

Aldehydes.—The method of Rowan (1) gave 6.28% average of 6 samples calculated as *n*-decylic aldehyde. Because Poore (2) reported 0.8% to 2.2% in California Valencia oil and 0.6% to 1.0% in Washington Naval Oil by Kleber's method (3) and because Schimmel (4), Finnemore (5) and Bennett (6) report lower percentages in different oils the method of Stillman and Reed (7) was used giving 5.55% average for 3 samples. To be certain of results the method of Bryant and Smith (8) was used giving 5.59% average of 3 samples. The bisulfite method (9) was found not suitable due to formation of solid material.

Esters.—The method of Seeker and Kirby (10) gave 0.65%, 0.67%, 0.74% esters calculated as linalyl acetate, average from lots by three producers.

Acid Value.—Method of A. O. A. C. (11) gave an average of 3.18.

Acetyl Value.—Method of Gildemeister and Hoffman (12) gave for 3 determinations 9.3 average.

Evaporation Residue.—Found by U. S. P. XI method: 0.1357, 0.1365, 0.1452, 0.1435-Gm. residue from 5.0-Gm. samples. Required: not less than 0.1 Gm.

Waxes.—No method could be found in the literature to determine the wax content, a knowledge of which is desirable to some dealers. After reviewing the literature on the dewaxing of petroleum oils the following method was arrived at. Mix 100 cc. of the oil with a solvent consisting of 30 cc. acetone, 45 cc. benzene and 45 cc. toluene. The mixture is chilled and the precipitated wax filtered through a tared Gooch crucible and dried in a vacuum desiccator over sulfuric acid until constant weight. To check the efficiency of this method, a sample of wax from orange peels was obtained from the U. S. Citrus Products Station, Winter Haven, Fla. Since oil of orange contains about 90% limonene, the wax was dissolved in pure limonene and treated as above. From three samples the reclaimed amount was 77.1%, 77.6%, 77.9%, av. 77.2%. Therefore results by this method must be divided by 77.2% or multiplied by the factor 1.29. This method of assay and calculation gave 0.04%, 0.05%, 0.05%, av. 0.05% wax.

Fractional Distillation.—Five hundred cubic centimeters of oil was fractionated at 20 mm. using a spiral screen packed column and still head described by Lecky and Ewell (13). Table I gives the results.

TABLE I.—FRACTIONATION OF OIL

Fraction	Vol., Cc.	B. P., ° C., 20 Mm.	B. P., ° C., 760 Mm.	d^{25}_{25}	n^{20}_{D}
1	10	60–62	...	....	1.4697
2	..	65–67	175	0.8443	1.4737
3	..	65–67	175	0.8443	1.4737
4	..	65–67	176	0.8443	1.4737
5	..	65–67	176	0.8441	1.4737
6	..	65–67	176	0.8439	1.4737
7	..	65–67	177	0.8438	1.4737
8	..	65–67	177	0.8438	1.4738
9	..	65–67	177	0.8434	1.4738
10	..	65–67	177	0.8442	1.4739
11	442.0[a]	A semicarbazone could not be prepared			
12	4.0	83	206	0.8273	1.4589
13	2.0	83	206	0.8273	1.4589
14	4.0	110	225	0.8872	1.4799
Residue	20.0	...	...	....	....
Loss	18.0	...	...	....	....

[a] Total fractions 2–11, inclusive, collected in same vessel.

IDENTIFICATION OF CONSTITUENTS

d-Limonene.—Fractions 2 to 11, inclusive, boiled within such a narrow range that they were combined. The physical properties closely agree with those recorded for *d*-limonene. It was further identified by its *α*-nitroso chloride, m. p. 102–103° C., *β*-nitrosochloride, m. p. 100–101° C., and tetrabromide, m. p. 103° C. The derivatives were made according to Parry (14). Fractions 1 to 11, inclusive, are no doubt *d*-limonene, 90.4% of the original oil.

n-Decylic Aldehyde.—Table II proves *n*-decylic aldehyde in fraction 12.

Citral.—Table III proves citral in fraction 14.

Alcohols.—The residue left after fractionation was saponified with alcoholic KOH. The alcohol was distilled off and the material extracted with ether. The ethereal solution was washed until neutral and dried over anhydrous sodium sulfate. A portion of it was treated with 3,5-dinitrobenzoyl chloride. A few crystals were obtained which melted at 50° C. The small amount was insufficient to recrystallize. The 3,5-dinitrobenzoate of nonyl alcohol melts at 52° C. and inasmuch as this alcohol has been found before in oil of orange the substance was probably *nonyl alcohol.*

TABLE II.—COMPARISON OF FRACTION 12 WITH *n*-DECYLIC ALDEHYDE

Property	Fraction 12	Recorded for *n*-Decylic Aldehyde
Color	Light yellow	Light yellow
Odor	Lemon odor	Lemon odor
B. p., 760 mm.	206° C.	207–209° C.
Sp. gr. $\frac{25}{25}$	0.8273	0.8280
n_D^{20}	1.4589	1.4297
Opt. rot.	Inactive	Inactive
Semicarbazone, m. p.	101° C.	102° C.
2,4-Dinitrophenylhydrazone, m. p.	104° C.	104° C.

Linalool has been previously found in oil of orange. It was identified in the remaining ethereal solution. After the ether was distilled off, a few cc. of an oil came over having a terpene odor. The next portion had a slight rose-like odor and the following properties: B. p., 180° C. at 765 mm.; $[\alpha]_D^{25}$ −12.8°; sp. gr. $\frac{25}{25}$ 0.8654; n_D^{20} 1.4683. Recorded for linalool b. p., 197–199° C. at 760 mm.; $[\alpha]_D^{25}$ −19.3°; sp. gr. $\frac{25}{25}$ 0.870–0.875; n_D^{20} 1.4668. The difference in properties, particularly the b. p., is no doubt due to traces of limonene and the alcohol is probably *linalool.*

TABLE III.—COMPARISON OF FRACTION 14 WITH CITRAL

Property	Fraction 14	Recorded for Citral
Color	Light yellow	Light yellow
Odor	Strong lemon odor	Strong lemon odor
B. p., 20 mm.	110° C.	118–119° C.
B. p., 760 mm.	226° C.	228–229° C.
Sp. gr. $\frac{20}{20}$	0.8872	0.8898
n_D^{20}	1.4799	1.4891
Opt. rot.	Inactive	Inactive
Semicarbazone, m. p.	161° C.	164–171° C.
Naphthocinchoninic acid derivative, m. p.	195° C.	197° C.
2,4-Dinitrophenylhydrazone, m. p.	107° C.	108–110° C.

Flavoring Properties of the Oil.—Aromatic Elixir U. S. P. XI was made with Florida Oil of Orange and with oils from two other geographical sources. When submitted to 10 different pharmacists labeled in code for tasting, 5 of them chose the elixir made with the Florida oil. The other 5 chose an elixir made with another oil. An elixir made with the third oil was not chosen by any of them.

Preparation of a Concentrate.—The well-known advantages of concentrated or terpeneless oils plus the fact that most processes for producing them are secrets stimulated the following trial process. Distillation and solubility in a hydroalcoholic solvent are no doubt key factors. Therefore it was determined what per cent hydroalcoholic solution would dissolve concentrates and not dissolve *d*-limonene; 81% alcohol appeared best. The process was begun by the Essential Oil Producers, Inc., using 6 gallons of oil of orange. Five gallons was distilled off as terpenes. From the remaining gallon a 200-cc. portion was distilled at 20 mm. in the fractionating column already mentioned; 108 cc. of limonene was collected. Column hold up 14.0 cc., residue 78.0 cc. The latter was treated with 1 L. of 81% alcohol. About 40 cc. of solid (probably waxes) and liquid were undissolved. The hydroalcoholic solution was separated and about 300 cc. of alcohol distilled off under partial vacuum. The remainder was placed in a separatory funnel and a salt solution added until the oil separated. After drying over anhydrous sodium sulfate, 25.0 cc. of a concentrate was obtained which had a very smooth, fine orange odor. The low yield could no doubt be raised by further study.

SUMMARY

The physical and chemical properties of Florida Oil of Orange have been determined. The oil meets the U. S. P. XI standards except for the index of refraction for the 10% distillate.

A method is given for determination of percentage wax in the oil.

Taste tests on Aromatic Elixir U. S. P. XI using Florida Oil of Orange proved that it was equal or superior to other oils.

A method is given for the preparation of concentrates.

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Florida Volatile Oils

V. *Illicium floridanum*, Ellis*

By A. W. Matthews† and P. A. Foote‡

Illicium floridanum, Ellis, family *Magnoliaceae*, is stated by Small (1) to be a shrub 2–3 M. tall found in swamps, low hammocks and on the coastal plain from North Florida to Louisiana and North Alabama. Various common names have been applied to it, among which may be mentioned Southern Star-Anise, Florida Stink-bush, Purple Anise, Poison Bay.

The genus *Illicium* is important because of the commercial use of the volatile oil which is obtained from the fruit of *Illicium verum*. This well-known species and *I. religiosum* are natives of China while two others, *I. floridanum* and *I. parviflorum*, are reportedly found in the southern states of North America. *I. parviflorum* has also been reported as occurring in other regions, notably Madagascar (2) and Anam (3), but *I. floridanum* would appear to be restricted to this continent.

The official, or Chinese Star-Anise (*I. verum*, Hooker), has been extensively investigated. The Japanese Star-Anise (*I. religiosum*, Siebold), because of the fact that the fruit resembles that of the true Star-Anise so closely that it is often mistaken for it or sold as an adulterant, has also received considerable botanical and chemical attention. S. Y. Chen (4) has compiled an extensive bibliography on it.

In 1901 Schlotterbeck and Eckler (5) stated that, in contrast to *I. verum* and *I. religiosum*, the American species have been practically neglected, a remark which would be almost equally true today. In 1885, Maisch (6) made a botanical study of *I. floridanum* and published a short description of the histological characters of the root, stem, leaf, capsule and seed along with several drawings. Later Schlotterbeck and Eckler carried out a more detailed study, which was likewise botanical in nature. To date no complete phytochemical study of *I. floridanum* appears to have been made nor have the constants of the volatile oil been reported. Recently Foote (7) examined a volatile oil obtained from the leaves of *I. pariflorum*, Michx. by steam distillation and reported a high safrol content (over 90%) with but traces of acids and esters.

Of much interest is the fact that some of the constants of *I. floridanum* closely approach those of *I. religiosum* (4, 8, 9).

EXPERIMENTAL

Distillation Studies of the Fresh Leaves.— To arrive at a quantitative estimate of the oil present in the

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leaf, the apparatus described by Middleton and Cocking (10) was used. In general, the procedure was as follows: 40 to 50 Gm. of the fresh leaf was added to about 400 cc. of water, previously brought to a boil in the heating flask. The flask was then connected to the condenser-receiver and was gently rotated until the water was again boiling freely. After 2 hrs. heating, the flame was removed and the volume of oil read. In Table I the results are given.

TABLE I.—COMPARISON OF YIELDS

Date	Source of Leaf	No. of Determinations	Av. Yield, %
Oct. 11, 1938	Gainesville	4	0.21
Feb. 7, 1939	West Florida	2	0.50
Mar. 13, 1939	Gainesville	2	0.19
July 10, 1939	Gainesville	3	0.16

In order to obtain additional oil from the leaf collected in Gainesville[1], 1650 Gm. of fresh leaf was distilled on October 25, 1938. Yield, 0.11%. The West Florida leaf was available in larger quantities. Approximately 10 Kg. of it was distilled in an all-metal still connected directly with the steam line. Yield, 0.335%, including that obtained by cohobation of weak distillates. For the purpose of comparison 1200 Gm. of this same leaf was distilled in an all-glass still, in which case the yield was 0.325%, also including cohobated oil. The latter distillations were conducted February 7 and 8, 1939.

The oil distilled from leaf collected in October was found to have d_{15}^{20} 0.8788; n_D^{20} 1.4723 and an ester value of 96.41, whereas the oil obtained in February from West Florida leaf had the following constants: d_{15}^{20} 1.0490; $[\alpha]_D^{25}$ −0.91°; n_D^{25} 1.5207; A. V. nil; E. V. 11.31; E. V. after acetylation 85.27 (Boulez method, 11). Upon carrying out an acetylation by the ordinary method, a value of only 46.6 was obtained.

The almost colorless West Florida oil gradually acquired a light yellow color. It possessed a characteristic pleasant and refreshing odor, although possibly less so than the oil distilled in October. When tested by sodium fusion, nitrogen and sulfur were found to be absent.

Phenols.—A 10-cc. sample was shaken in a cassia flask with solution of KOH and allowed to stand in contact with it overnight. No absorption.

Aldehydes and Ketones.—The oil gave a faintly positive test with Tollen's reagent but was negative with Schiff's reagent and with the test of Rodionow and Korolew (12). A small sample was examined with semicarbazide reagent by the micromethod, and a larger sample treated with sodium bisulfite. Both failed to give an addition product.

Saponification and Fractionation.—A preliminary micro boiling-point determination indicated a high boiling range. Fifteen cubic centimeters of the sample was then heated on a water bath for 1 hr. with 15 cc. of 0.5 *N* alcoholic potash, after which the greater part of the alcohol was distilled off, washed and dried over anhydrous sodium sulfate. It was fractionated under 15 mm. pressure with the results shown in Table II.

TABLE II.—FRACTIONATION

Fraction	Volume, Cc.	B. P., °C., 15 Mm.	B. P., °C., 760 Mm.	d_{15}^{20}	n_D^{20}
1	0.5	62	190	.....	1.4650
2	0.75	73–74	242	0.9515	1.5068
3	2.5	121	270	1.1114	1.5385
4	4.3	126–130	275	1.1331	1.5399
Residue	1.0				
Total	9.05				

Fraction 1.—Possessed the characteristic odor of the original sample. When cooled to a low temperature with dry ice (below −30° C.), the liquid became exceedingly viscous but without crystal formation. The total amount available was used in an unsuccessful attempt to prepare and separate a phenylurethane.

Fraction 2.—The odor appeared to be somewhat different from that of the above-mentioned fraction. When cooled to a low temperature, there was a separation of crystals following which the whole set into a solid mass. The melting point appeared to be approximately 0° to 2° C. No phenylurethane could be obtained.

Fraction 3.—This was almost devoid of odor and congealed at −17° C. to such an extent that no liquid could be poured off. The melting point in this case was approximately 5° to 8° C. One cubic centimeter was examined for safrol by the method employed successfully by Foote (7), but no piperonylic acid was obtained. When this same procedure was applied to a known sample of safrol, piperonylic acid (m. p. 227–228° C.) was obtained.

Fraction 4.—This fraction was likewise almost devoid of odor, and the constants differed but slightly from fraction 3. The congealing point was also identical, and the melting point was slightly higher (8–10° C.). Some liquid was at first separated during the congealing, but when again chilled, this also solidified and further examination showed it to have constants not appreciably different from the original fraction. Two cubic centimeters was tested for isosafrol by the method of Wallach and Müller (13). No isosafrol nitrite could be separated.

DISCUSSION

It is apparent that the properties of volatile oil present in the leaf of *I. floridanum* vary at different seasons of the year. In October, again in March and in July the yield was approximately 0.20%. Variations

[1] The authors gratefully acknowledge the coöperation of Dean H. Harold Hume of the College of Agriculture who supplied the plant material for this investigation.

from this figure reported in Table I must be regarded as within the limit of error in reading the amount of oil in the measuring arm of the receiver. In February the yield of oil had apparently risen to 0.50%. It must be said, however, that there are two factors which may have some bearing on this high yield. In the first place, this leaf (collected in West Florida) contained a small proportion of twigs and, second, distillation was not carried out until several days after collection. The distillation yields also indicated that by the methods of steam distillation, such as might ordinarily be employed in the laboratory, all the oil present in the leaf is not recovered. When 0.21% was present, only 0.11% was obtained; and when 0.50% was present, a maximum of 0.335% was recovered, even when the weak distillate was cohobated.

More striking than the seasonal variation in yield of oil are the differences between the physical properties of the oils distilled in October and in February. During this interim the specific gravity underwent a transition from 0.8788 to 1.0490, and the refractive index from 1.4723 to 1.5207. The possibility of an environmental factor in this regard was checked. As soon as the increase in density was noted, a small sample of leaf was collected from the Gainesville source and distilled (March 13, 1939) in Middleton and Cocking's apparatus. Again the oil was heavier than water.

Coincident with the above noted change in physical properties, there was a decline in the ester value from 96.41 in October to 11.31 in February. The limited sample of the former oil did not permit a further comparison, but with the latter an ester value after acetylation (11) of 85.27 was noted (46.6 when determined by the regular method). This last observation apparently points to the possibility of a tertiary alcohol, and it is to be noted that linalool has been reported (8) as a constituent of the oil of *I. religiosum*. The observed boiling point and refractive index (b. p. 198–199° C. per 760 mm., n_D^{20} 1.4668) of the very small fraction 1 correspond closely to those reported (14) for linalool, but no phenylurethane could be obtained for confirmation.

The specific gravities and refractive indices of fractions 3 and 4 are in good agreement with those of safrol, for which Eykman (15) reported d_{15} 1.0960 and n_D 1.5420. It was also noted that, when solidified, these fractions melted at 5° to 10° C. (Eykman gives 8° C. for safrol.) In view of the fact that safrol has been reported (7, 8, 16) as a constituent of the closely related oils *I. parviflorum* and *I. religiosum*, it is a likely possibility here. However, the boiling range over which these two fractions were obtained is higher than that usually reported for safrol, and furthermore oxidation by alkaline permanganate failed to yield piperonylic acid. The higher density of fraction 4, together with the fact that the oil was boiled with alcoholic KOH prior to fractionating, suggests the possible presence of isosafrol in this fraction, but the refractive index does not bear this out. Furthermore, when tested for isosafrol by the method of Wallach and Müller, no nitrite was obtained. However, there is distinct evidence of the presence in the 2 fractions of some substance of higher boiling point and density than safrol and which refractionation of a larger sample might definitely establish.

Likewise, there is some doubt as to the existence of a true fraction in between these high boiling fractions and the fraction of 190° C. The refractive index of this portion of the distillate is intermediate between fraction 1 and fraction 3, and the specific gravity was considerably less than that of fraction 3. However, behavior under cooling was suggestive of the latter fraction.

On the basis of the physical evidence presented it would appear that in *I. floridanum* a high boiling point, high gravity constituent or constituents develop in the oil with the approach of the fruiting stage, apparently at the expense of the esters present in the oil earlier in the season.

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The Pharmacological Action of Methylamino-iso-octene (Octin)

I. The Action on Blood Pressure*

By R. P. Ahlquist†‡

Methylamino iso-octene (Octin) is an unsaturated aliphatic amine. Octin base is a colorless, oily fluid forming water-soluble salts with acids. The hydrochloride was used throughout this work.

Octin has been recommended for use as a smooth muscle spasmolytic. Small doses have been shown to relax the musculature of the gastrointestinal tract (1, 2, 3).

That Octin has a definite pressor action when administered intravenously to experimental animals has been shown by many authors (1, 2, 3, 4), but there is some disagreement as to its cause. Adrenergic stimulation resulting in vasoconstriction and cardiac stimulation, or a direct irritation of the blood vessels, have both been suggested as the cause of the increased blood pressure. A preliminary fall in blood pressure has been noted by most of the workers, but the significance or extent of this hypotension has not been fully determined.

This paper is concerned with the effect on blood pressure in anesthetized animals of single doses and repeated doses of Octin administered intravenously. The effect of preliminary administration of cocaine has also been determined.

EXPERIMENTAL

Dogs weighing from 7 to 23 Kg. were anesthetized with ether. Blood pressure was recorded by the usual method from the common carotid artery by means of either a mercury or a membrane manometer. Respiration rate was recorded in some of the experiments by means of a pneumograph around the chest and a tambour. The cervical vagi were sectioned in all of the experiments. The femoral vein was cannulated and connected with rubber tubing to a pressure bottle containing warm physiological saline solution. The drugs were injected into the tubing and washed into the vein with about 10 cc. of the saline solution.

A few rabbits were anesthetized with sodium barbital and the blood pressure recorded as above. The drug was injected into the marginal ear vein.

The Octin solution was freshly prepared for each experiment by diluting a 10% stock solution with physiological salt solution to a concentration of 10 mg./cc. The cocaine solution was freshly prepared in a concentration of 25 mg. of cocaine hydrochloride/cc.

The kymograph was set for a speed of about 1 cm. per minute and a normal record was taken for about 15 min. before any drug was administered. A marker was set to record this normal pressure throughout the entire experiment. Decreases in pressure were measured from the pressure at the time of injection to the lowest point recorded. Increases in pressure were measured from the normal pressure line to the highest point recorded. All of

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the reproduced pressure tracings were made with the mercury manometer. The upper line is the mean blood pressure, the middle line the normal pressure and the lower line the pressure base line with time signals at 1-min. intervals.

RESULTS

Single doses of Octin ranging from 1 to 10 mg. (all doses are in terms of mg./Kg.) produced a slight fleeting drop in pressure followed by a rapid increase in pressure measuring from 80 to 160 mm. of mercury. The pressure then fell gradually over a period of 15 to 90 min. to the normal level. The duration of action was more or less proportional to the dose administered. Figure 1 (*A*) shows part of a typical blood pressure curve produced by a single dose of Octin.

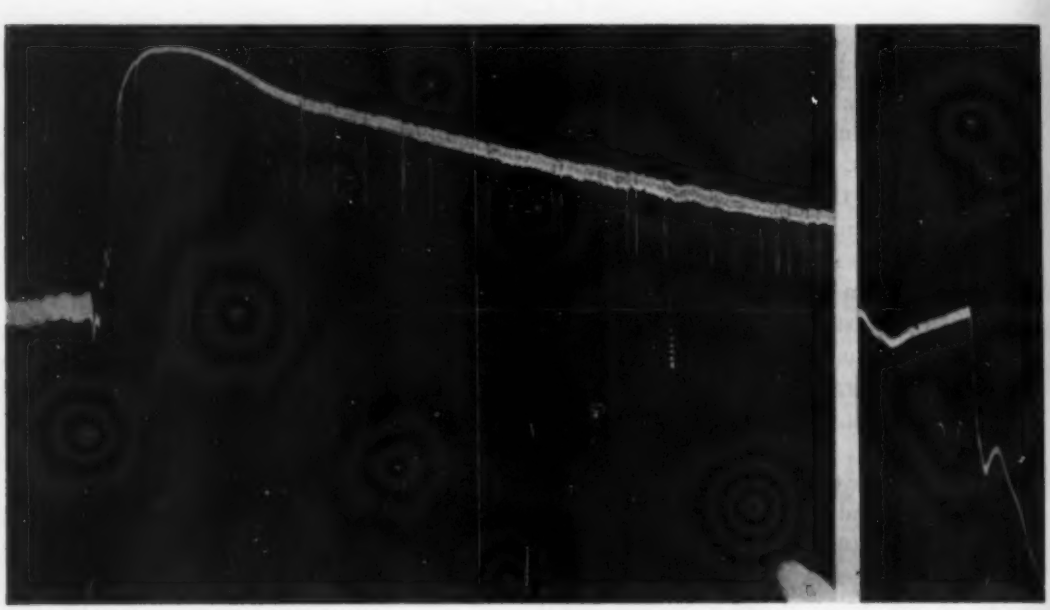
If a second dose of Octin was given after the first injection and the total dose approached 10 mg., death was produced in some cases. Figure 1 (*B*) shows the results of repeating the original dose of 5 mg. This type of response is typical of all of the deaths produced by Octin. Table I gives the results of the administration of various single and double doses of Octin to 5 different dogs.

Intravenous doses of 1 to 5 mg. in rabbits produced results similar to those in the dog, differing only in that the pressor response was smaller in proportion to the dose given.

In order to determine the effect of repeated administration, a dose of 2 mg. of Octin was administered intravenously to a dog. When the pressure had returned to about normal, this dose was repeated. This procedure was continued until 3 or 4 doses had been given. The pressure drop became more marked with every dose subsequent to the first injection.

TABLE I.—PRESSOR RESPONSE TO VARIOUS DOSES OF OCTIN ADMINISTERED INTRAVENOUSLY TO DOGS

Dog No.	Dose, Mg./Kg.	Pressor Response Mm. of Mercury	Duration of Action, Min.
1	5	112	50
	5	Death	..
2	2	100	23
3	1	95	15
4	1	110	30
	10	Death	..
5	10	160	90
	10	Death	..



1 2

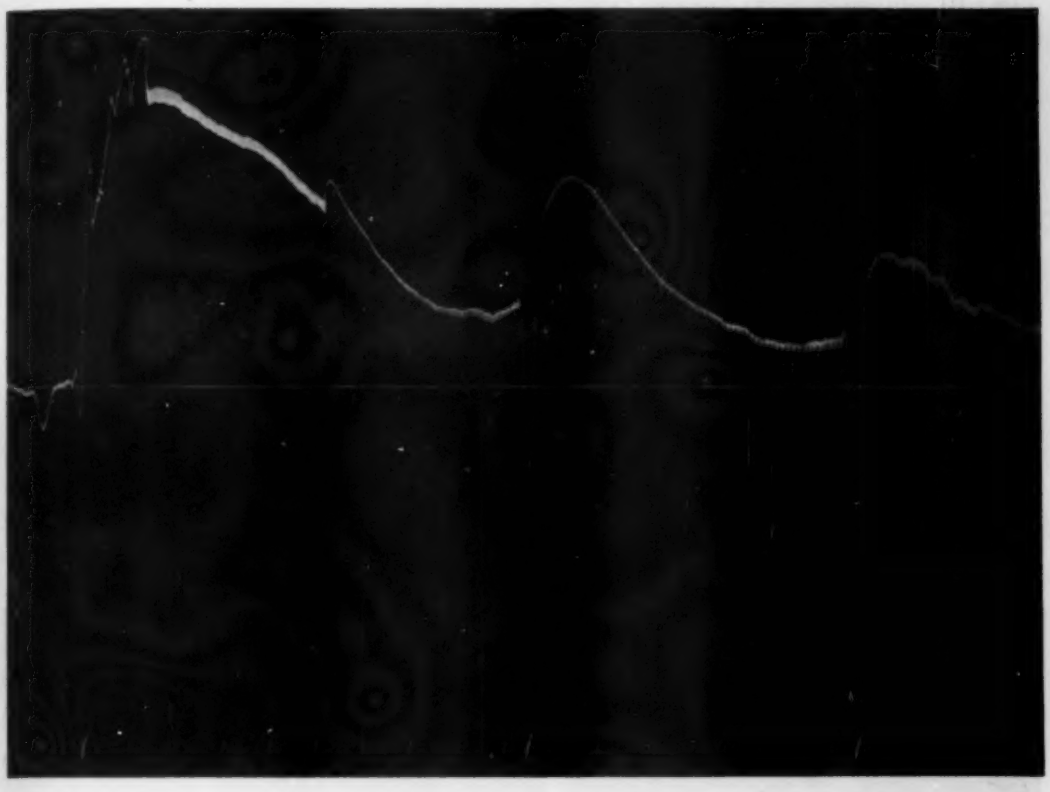
A *B*

Fig. 1.—Blood Pressure Tracing Produced by Octin Administered Intravenously to a Dog. Dog number 1, female, 20.5 Kg. (*A*-1) Octin 5 mg.; (*B*-2) Octin 5 mg. 60 min. after *A*-1.

In some of these experiments the heart rate was measured by means of the membrane manometer recording on a fast kymograph. The rate was determined during a normal pressure tracing and during the hypotensive and hypertensive stages. The only measurable difference in rate was observed during the hypertension produced by the original dose. The rate here was slightly increased.

The membrane manometer record also showed there was little or no change in pulse pressure during the preliminary hypotension. Any change noted

at all was a slight decrease in pulse pressure. The cause of this pressure drop has not been fully established, but, as will be pointed out in the discussion, it is probably due to direct myocardial depression. The decreased pressor response is similar to that produced by sympathomimetic amines such as ephedrine. Figure 2 shows the results of three injections of 2 mg. of Octin. This record is typical of all produced. Table II gives the results of repeated doses of Octin in 5 different dogs.



1 2 3

Fig. 2.—Blood Pressure Tracing Produced by Three Repeated Intravenous Doses of Octin. Dog number 6, female, 7 Kg. Octin 2 mg., administered at 1, 2 and 3.

The effect of cocaine on the pressor response of Octin was determined in a series of experiments on dogs. The procedure was similar to that used in determining the effect of repeated doses of Octin but with various doses of cocaine hydrochloride being given just before the Octin. Doses of 1 to 10 mg. of cocaine were used and 2 mg. of Octin was given. Figure 3 shows a typical pressure tracing produced by Octin and cocaine. Table III gives the results obtained in 10 experiments on 4 dogs. The predicted pressor response is the average increase in pressure expected with each repeated dose of Octin (Table II). The results indicate that cocaine has no significant effect on the Octin pressor response.

TABLE II.—BLOOD PRESSURE CHANGES PRODUCED BY REPEATED DOSES OF OCTIN IN ANESTHETIZED DOGS

	Repeated Dose of 2 Mg. of Octin							
	I		II		III		IV	
Dog. No.	Dec.[a]	Inc.[b]	Dec.	Inc.	Dec.	Inc.	Dec.	Inc.
6	14	130	60	74	60	46	..	...
7	12	152	52	30	60	14	86	— 2
8	20	80	36	50	50	24	44	36
9	14	110	44	56	56	40	..	...
10	10	122	45	60	50	36	..	...
Average	14	119	45	64	57	32	65	17
Range	..	±39	..	±34	..	±18	..	±19

[a] Preliminary decrease in blood pressure in mm. of mercury.
[b] Increase in blood pressure in mm. of mercury (pressor response).

The respiratory changes recorded in some of the experiments were not marked. In general the respiration rate varied inversely with the blood pressure changes produced by the doses of Octin used, and appeared to be governed by this pressure entirely. For this reason no records of respiration rate are included in this report.

TABLE III.—BLOOD PRESSURE CHANGES PRODUCED BY ALTERNATE DOSES OF OCTIN AND COCAINE IN ANESTHETIZED DOGS

Dog No.	Cocaine HCl, Mg./Kg.	Pressor Response to 2 Mg. of Octin, Mm. of Mercury: Experimental	Predicted[a]	Difference
11	..	110	119	9
	1	56	64	8
	5	Death	...	..
12	..	86	119	33
	2	52	64	12
	5	22	32	10
13	..	140	119	21
	2	60	64	4
	5	40	32	8
	5	Death	...	..
14	..	124	119	5
	10	54	64	10
	..	32	32	0
	..	2	17	15

[a] Average pressor response to repeated doses of Octin See Table II.

DISCUSSION

Octin as shown by its action on blood pressure in anesthetized dogs appears to be a sympathomimetic amine of the ephedrine type. Octin produces a sustained pressor response; it appears to stimulate the heart directly as shown by a slight increase in heart rate; it produces decreasing pressor responses to repeated doses; and cocaine has no marked effect on its pressor action. Other workers (1, 2) have shown that it relaxes the musculature of the intestine, relaxes the bronchial muscle somewhat, produces mydriasis and has other sympathomimetic effects.

Of significant interest is the increasing hypotension produced by repeated doses of Octin. It was noted that during this pressure fall there was no significant change in heart rate and there was little change in pulse pressure. The fact that pulse pressure was not increased is indicative that general vasodilation was not the cause of the hypotension. Central vagal stimulation is ruled out because both vagi were sectioned. As Pilcher (5) has pointed out, a decrease in blood pressure with decrease in pulse pressure is due to either cardiac weakening or decreased blood volume. As no evidence of diminished blood volume was seen, the hypotension is therefore probably due to a direct, temporary myocardial weakening. This hypotensive effect is then overcome by the pressor action of the drug resulting in

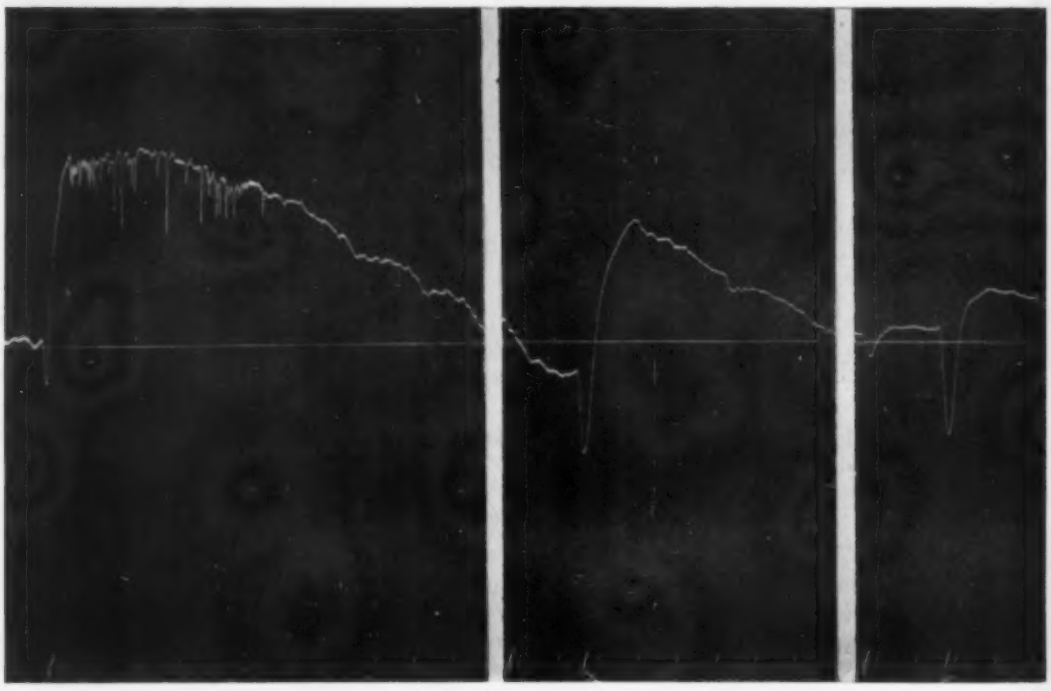


Fig. 3.—Blood Pressure Tracings Produced by Alternate Doses of Octin and Cocaine Hydrochloride. Dog number 12, female, 13.5 Kg. (*A*-1) Octin 2 mg.; (*B*-2) Cocaine 2 mg.; (*B*-3) Octin 2 mg.; (*C*-4) Cocaine 5 mg.; (*C*-5) Octin 2 mg. About 15 min. elapsed time between records.

the hypotensive stage. The heart either becomes more sensitive to the inhibitory effect of the drug or the decreasing pressor action allows the pressure to fall more with each repeated dose subsequent to the original dose.

SUMMARY

Octin when administered intravenously to anesthetized animals produces a sustained increase in blood pressure similar to the sympathomimetic amines.

Repeated doses produce decreasing pressor responses and increasing preliminary pressure drops. Evidence is given that the hypotension is due to direct myocardial depression.

Cocaine has no significant effect on the pressor response produced by Octin.

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Salmon Protein as a Source of Histidine, Arginine, Lysine, Cystine, Tyrosine and Tryptophane*

By Louis Arrigoni† and Louis Fischer‡

The amino acids have long been recognized as the hydrolytic products of protein and the ultimate form in which it is absorbed from the small intestine. Only recently, however, have researches into the therapeutic properties of these compounds been undertaken—and with illuminating results. Several of these compounds have been found to be of considerable value in the treatment of organic and deficiency diseases.

For instance, glycocoll has been used effectively in the treatment of myasthenia gravis, adiposis dolorosa, hepatic diseases, cardiac stasis and in the detection of liver damage (1, 2, 3). Tryptophane, as such or, better still, in combination with histidine, copper and iron, has been found to elevate the iron reserves in secondary anemia, and when given in small doses, to shorten the healing time of wounds on dogs (4, 5). Glutamic acid hydrochloride has become quite important as a therapeutic agent in the treatment of achlorhydria, pernicious anemia and other conditions of the stomach, characterized by a deficiency of hydrochloric acid (6). It has also given promising results in the treatment of various muscular weaknesses, dystrophies and atrophies (7). Cystine, the amino acid found most abundantly in hair, nails and other keratinized tissues, has been investigated in regard to its value in stimulating hair growth, with the discovery that cysteine, a reduction product of cystine, was able to induce the growth of hair on hairless rats (8). Researches by Jacobsen and Lubborow (9) would seem to indicate that one of the factors in liver extract which renders it therapeutically active in the treatment of pernicious anemia is tyrosine. Other amino acids have found limited use in various therapeutic manifestations, and future experimentation undoubtedly will bring to the surface a great deal more information which will serve both to corroborate and enlarge upon the conclusions of the past researches and to set forth new aspects and applications.

The knowledge that these compounds were assuming such an important place in medicine, together with the fact that at present some of these compounds are so expen-

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sive as to remove them from the sphere of the average patient, prompted this investigation. The ultimate goal was directed toward finding a less expensive source of raw materials which would, in turn, reduce the cost of the finished product. It is with this thought in mind that the authors decided to investigate salmon protein as a source of amino acids for this expanding need in therapeutics. Providing that salmon protein contains these amino acids, there is a possibility that the waste material from the salmon industry could be utilized as a source of these essential substances.

EXPERIMENTAL

In all cases the amino acids were isolated from the dried, defatted muscle protein of canned pink salmon, *Oncorhynchus gorbuscha*.[1]

ISOLATION OF THE DIAMINO ACIDS—HISTIDINE, ARGININE AND LYSINE

In 1900 Kossel and Kutscher (10) published their method for the quantitative estimation of the diamino acids—histidine, lysine and arginine. This procedure was the earliest to give approximately accurate quantitative results for these constituents, and, with modifications, is still one of the best means for the estimation of these compounds in protein.

This procedure depends upon the precipitation of arginine and histidine as their silver salts, and their separation by differences in solubility at different *p*H levels, and the subsequent precipitation of lysine first by phosphotungstic acid and ultimately by picric acid. This method was modified in 1903 by Kossel and Patten (11), in 1906 by Kossel and Pringle (12) and in 1915 by Osborne, Van Slyke, Leavenworth and Vinograd (13).

The method used by the authors is a combination of Kossel and Kutscher's original procedure and the modifications suggested by the above-mentioned workers. The essential steps in the procedure employed for the isolation and estimation of the diamino acids are as follows: The dried, defatted salmon protein was hydrolyzed with 25% sulfuric acid until the biuret reaction gave negative results. The sulfuric acid was removed with baryta water, the barium sulfate filtered off and the ammonia removed from the filtrate by heating with an excess of barium carbonate. At this point the solution was made slightly acid with sulfuric acid and decolorized with norit. The clear solution, freed from ammonia and containing a small amount of sulfuric acid, was treated with a slight excess of silver nitrate which served to precipitate the histidine and arginine as their silver salts. The mixture was saturated with finely divided barium hydroxide and the precipitated silver salts of histidine and arginine filtered off and washed with baryta water. The filtrate contained the lysine. The precipitated silver salts of histidine and arginine were suspended in dilute sulfuric acid and decomposed with hydrogen sulfide. The silver sulfide was filtered off and the sulfuric acid precipitated with baryta and removed by filtration.

Isolation and Quantitative Determination of Histidine as the Picrolonate.—The separation of histidine from arginine was accomplished by the method proposed by Osborne, Leavenworth and Brautlecht (14), in which the greater portion of the histidine is removed by precipitation with mercuric sulfate (10% $HgSO_4$ in 5% sulfuric acid). The histidine-mercuric sulfate precipitate so obtained was suspended in water and decomposed with hydrogen sulfide. The filtrate obtained, after removing the mercuric sulfide, was freed from sulfuric acid with baryta water and barium nitrate, and the histidine precipitated as the silver salt, as described above, filtered, washed and set aside.

The filtrate from the histidine-mercuric sulfate was freed from mercury and sulfuric acid in the usual manner. The resulting filtrate was acidified with nitric acid and treated with a slight excess of silver nitrate. At this point the reaction of the solution was adjusted to exactly *p*H 7.0 using baryta and a Beckmann *p*H meter. At this *p*H the arginine silver is soluble and the histidine silver is insoluble, according to Vickery and Leavenworth (15), thus making it possible to obtain a very good separation of the 2 compounds. The histidine silver salt was filtered off and washed with baryta. The filtrate contained the arginine as the soluble silver salt.

The 2 precipitates of histidine silver were combined, suspended in hot water, acidified with sulfuric acid and decomposed with hydrogen sulfide. The silver sulfide was filtered off and the excess hydrogen sulfide removed by boiling. A nitrogen determination was performed on an aliquot of the histidine solution by means of the Van Slyke amino nitrogen apparatus. From the amount of amino nitrogen present the concentration of histidine in the solution was estimated. From this figure the amount of picrolonic acid necessary to completely precipitate the histidine was calculated.

The histidine solution freed from both sulfuric acid and baryta was concentrated to about 30 cc. and treated with picrolonic acid slightly in excess of the amount necessary to completely precipitate it. The precipitate of histidine picrolonate was filtered off after 3 days. The salt was thoroughly washed with water, dried, weighed and the amount of histidine calculated. The yield of pure histidine was 1.55% of the dried, defatted protein (see Table I).

Estimation and Isolation of Arginine as the Picrolonate.—The filtrate containing the arginine silver was precipitated by saturating it with barium hy-

[1] The authors wish to express their gratitude to the National Canners Association of Seattle, Wash., who so generously contributed the salmon used in this investigation.

droxide; the silver salt of arginine was filtered off and washed free from nitrates. The precipitate was suspended in water, acidified, decomposed with hydrogen sulfide and the precipitated silver sulfide and barium sulfate removed by filtration.

The filtrate was freed from sulfuric acid with baryta, filtered and evaporated to about 30 cc. A slight excess of picrolonic acid, calculated from an amino nitrogen determination of the filtrate, was added and the yellow crystals of arginine picrolonate filtered after standing 3 days. The crystals were washed thoroughly with water, dried, weighed and the amount of arginine calculated. The yield of pure arginine was 4.62% of the sample taken for analysis (see Table I).

Estimation and Isolation of Lysine as the Picrate.—The filtrate from the precipitate of the silver salts of arginine and histidine, containing the lysine, was acidified with sulfuric acid and freed from silver with hydrogen sulfide. The filtrate from the precipitates of silver sulfide and barium sulfate was evaporated to about 500 cc. Sulfuric acid was then added until the solution contained 5% of this acid and the lysine completely precipitated by the addition of phosphotungstic acid. After 24 hrs. the precipitate of lysine phosphotungstate was filtered off and thoroughly washed with 5% sulfuric acid.

The lysine phosphotungstate was made into a uniform suspension with water and poured into boiling water. A hot saturated solution of baryta was added until the solution was strongly alkaline and contained a large excess of baryta. The precipitate of barium phosphotungstate was filtered and boiled several times with baryta, then with water and filtered. The alkaline filtrate was freed from baryta with carbon dioxide, concentrated, filtered again and evaporated on the water bath nearly to dryness. Water was then added, the barium carbonate removed by filtration and the filtrate, once more evaporated and made up to volume with water. The amino nitrogen was determined with the Van Slyke apparatus on a very small aliquot of the total solution.

The remaining solution was evaporated to dryness, treated with a small quantity of alcohol and the lysine completely precipitated with a saturated solution of picric acid in alcohol. After 24 hrs. this precipitate was filtered and recrystallized 3 times from boiling water.

The last portions of lysine in the mother liquor from the picrate were obtained by acidifying the solution with sulfuric acid, extracting the picric acid with ether, precipitating lysine as the phosphotungstate and repeating the above process for obtaining lysine picrate.

The yield of pure lysine, calculated from the weighed lysine picrate, was 6.22% of the total bulk of salmon protein taken for examination (see Table I).

TABLE I.—PER CENT OF CERTAIN AMINO ACIDS IN SALMON PROTEIN

Amino Acid	Protein Taken for Analysis, Gm.	Compound Isolated	Nitrogen in Isolated Compound, % Calculated	Nitrogen in Isolated Compound, % Theoretical	Amino Acids Recovered from Salmon Protein, %
Histidine	105	Histidine Picrolonate	23.52[a]	23.39	1.55
Arginine	105	Arginine Picrolonate	25.69[a]	25.56	4.62
Lysine	105	Lysine Picrate	18.54[a]	18.66	6.22
Cystine	150	Cystine	11.53[b]	11.65	1.05
Tyrosine	150	Tyrosine	7.85[b]	7.74	0.86
Tryptophane	200	Tryptophane	6.94[b]	6.96	0.97

[a] Nitrogen determined by Kjeldahl-Gunning-Arnold method.
[b] Nitrogen determined by means of Van Slyke amino nitrogen apparatus.

ISOLATION AND SEPARATION OF CYSTINE AND TYROSINE

Cystine, like tyrosine, is almost insoluble in neutral aqueous solution. They differ in this respect from all other amino acids, and this characteristic of the 2 is made use of in their isolation and determination in protein hydrolysates. Cystine and tyrosine, however, resemble each other so closely in their solubility in water, alkaline and acid solutions that their separation is rendered very difficult.

Folin's (16) method was used for the isolation and separation of cystine and tyrosine from salmon protein; the only modification instituted was the use of an accurate potentiometer type *p*H meter for the adjustment of the reaction of the hydrolysate to the isoelectric point of cystine (*p*H 4.3) instead of using congo red indicator as in the original procedure.

Procedure for Isolation of Cystine.—The dried, defatted protein was reluxed on a sand bath with concentrated hydrochloric acid until the biuret reaction was entirely negative. The hot solution was then filtered to remove the humin substances.

To the resulting acidic solution of the amino acids, solid sodium acetate was added until the *p*H was exactly 4.3. This solution of *p*H 4.3 was allowed to stand at room temperature for 3 days, then the dark gummy precipitate, consisting of crude cystine, was filtered off and washed with cold water. The filtrate containing the tyrosine was set aside and treated as described later.

The crude cystine was dissolved in 3% hydrochloric acid and the solution decolorized with norit. The

warm clear filtrate was treated with sodium acetate until the *p*H was again 4.3. The solution was set aside, and, after 3 days, the cystine crystals were filtered off and washed with water. The cystine was recrystallized as described above, then dried and weighed. The final product gave a negative Millon's test indicating the absence of tyrosine. The yield of cystine was 1.05% of the total bulk taken for examination (see Table I).

Procedure for Isolation of Tyrosine.—The filtrate from the crude cystine precipitate, containing the tyrosine, was diluted with about half its volume of water and then adjusted to *p*H 5.7 with 25% sodium hydroxide. This solution was set aside in a cool place for 7 days and then the resulting precipitate, consisting of crude tyrosine, was removed by filtration. This precipitate was dissolved in 3% hydrochloric acid and the resulting solution decolorized with norit. The warm, clear filtrate was treated with sodium acetate until the *p*H was about 4.5 and then the solution adjusted to a *p*H of 5.7 with 10% sodium hydroxide. The solution was again set aside for 7 days, the precipitated tyrosine filtered off, and, after recrystallizing as described above, the resulting tyrosine was dried and weighed. The final product gave a negative test with Sullivan's reaction, using sodium-β-naphtho-quinone-4 sulfonate, sodium cyanide and sodium sulfite, indicating the absence of cystine.

The yield of the tyrosine was 0.86% of the original salmon protein taken for examination (see Table I).

ISOLATION AND ESTIMATION OF TRYPTOPHANE

Tryptophane was first shown to be a constituent of protein by Hopkins and Cole (17), who in 1901 isolated 1.5% from casein. Their method revolved largely around the use of mercuric sulfate as the precipitating agent. They treated the protein hydrolysate with the mercuric sulfate reagent which precipitated the tryptophane, tyrosine and cystine. The tyrosine mercury complex was removed by washing the precipitate with 5% sulfuric acid in which it is soluble. The precipitate containing the cystine and tryptophane mercury was decomposed with hydrogen sulfide and the two separated by fractional precipitation with the mercuric sulfate reagent. The cystine precipitated first.

The above-mentioned method of Hopkins and Cole combined with Dakin's (18) butyl alcohol extraction procedure was employed by the authors in the isolation and determination of tryptophane in salmon protein.

Procedure for the Isolation of Tryptophane.—The dried, defatted salmon protein was placed in 2 L. of 1% sodium carbonate solution with 6 Gm. of trypsin (1–110) and digested at 37°C. The enzyme was added in 2 portions, 4 Gm. at the onset and 2 Gm. at the end of the third day of digestion. The mixture was kept aseptic by the addition of 2 Gm. of sodium fluoride and 50 cc. of toluene.

The digestion was continued for 8 days at which time the maximum concentration of tryptophane in the hydrolysate was obtained as indicated by the addition of bromine water.

The resulting solution was heated to 80° C., cooled and filtered. To the filtrate concentrated sulfuric acid was added until the concentration of the solution was approximately 5% by volume. The mercuric sulfate reagent (prepared by dissolving 10% mercuric sulfate in 5% sulfuric acid) was added in excess, *i. e.*, enough to bring about precipitation and then an additional 25 cc./L. of the mixture. The solution was then allowed to stand for 48 hrs. when a heavy lemon-yellow precipitate settled out. This was filtered and washed with 5% sulfuric acid (in which the mercury compound of tyrosine is soluble) until the washings gave a negative test (brownish yellow coloration) with Millon's reagent, which indicated that all of the mercury compound of tyrosine had been removed from the precipitate.

The washed mercury precipitate was suspended in about 400 cc. of water and decomposed with hydrogen sulfide. The decomposition proceeded very slowly; consequently, after complete saturation of the solution with hydrogen sulfide, the mixture was heated on a water bath for several hours and again saturated with the gas. To insure complete decomposition of the mercury compounds of tryptophane and cystine, the precipitate, after filtration, was again suspended in water and treated with hydrogen sulfide. Finally the mercuric sulfide was filtered off and the combined filtrates freed from the hydrogen sulfide by boiling for a short time.

The solution containing the tryptophane and cystine was freed from sulfuric acid by adding sufficient barium hydroxide solution to exactly precipitate it as barium sulfate, and the resulting solution was concentrated under reduced pressure to about 50 cc. It was then extracted with butyl alcohol in a Palkin extractor. At the end of the first hour the tryptophane began to settle out on the sides of the extraction flask, and in 12 hrs. the extraction was assumed to be complete as the residual aqueous solution gave a negative glyoxylic acid reaction.

The crystals of tryptophane which settled out from the butyl alcohol were purified by redissolving them in a minimal quantity of hot water, adding an equal volume of 95% alcohol, decolorizing the solution with norit and filtering while hot. On concentration of this filtrate, with the occasional addition of alcohol, the fine white crystals of tryptophane settled out. These crystals were recrystallized from 75% alcohol, dried and weighed. The yield of the tryptophane was 0.97% of the dried, defatted protein (see Table I).

DISCUSSION

It should be pointed out that the values obtained in the foregoing experiments for the percentage composition of the 6 amino acids studied is not a true measure of the content of each in salmon protein since, as in all isolation and purification procedures, some of the compounds are inevitably lost.

These values should be regarded as the upper limit or maximum that can be expected to be recovered by the isolation procedures used if this line of endeavor were undertaken commercially using salmon muscle protein as the source.

In all probability, improvements and modifications of the above procedures would lead, in some cases at least, to larger recovery of the amino acids. In fact, several methods which have been proposed are reported to give better results in the isolation of several of the amino acids studied by the authors. For example, Vickery and Leavenworth (15, 19) have reported that they obtained higher values in the isolation of histidine and arginine if they were precipitated as the dinitronaphthol-sulfonates rather than as the picrolonates. Harris (20) also has suggested a method which he claims gives a larger recovery of cystine from protein hydrolysates. The method depends upon the precipitation of cystine with mercuric sulfate reagent, followed by the decomposition of the mercury precipitate with hydrogen sulfide and precipitation of the resulting cystine with copper hydroxide.

Even though these methods do result in a larger recovery of the amino acids for which they are designed, they have the disadvantage of not being as readily applicable to commercial production of these compounds because of the more extensive operations required for the isolation.

The butyl alcohol extraction method developed by Dakin (18), which was used in the preparation of tryptophane by the authors, is, perhaps, the procedure that could be most efficiently and effectively converted into a feasible means, at least as the initial step, in the commercial preparation of the amino acids.

By this means, the products of a protein hydrolysate may be divided without serious loss into 5 groups composed of chemically similar compounds which facilitates the isolation and determination of the amino acids in each group.

Using Dakin's method most of the amino acids can be isolated from the same hydrolysate, whereas in the foregoing experiments 3 different samples of protein had to be used in the preparation of the 6 amino acids. This economy of material and labor would certainly decrease the cost of production and is worthy of investigation.

Work planned by the authors, for the future, on the lines discussed above might possibly bring to light more of the practical aspects of the problem of preparation of therapeutically important amino acids from salmon protein.

SUMMARY AND CONCLUSIONS

The 6 amino acids, histidine, arginine, lysine, cystine, tyrosine and tryptophane, all of which have been found to be useful in therapeutics, have been isolated and determined quantitatively in pink salmon protein. They were found present in the following amounts, expressed as per cent: histidine 1.55, arginine 4.62, lysine 6.22, cystine 1.05, tyrosine 0.86 and tryptophane 0.97.

Histidine, arginine and lysine were isolated from the hydrolytic products of 25% sulfuric acid on protein; cystine and tyrosine were recovered from the concentrated hydrochloric acid hydrolysate of the protein; and tryptophane was prepared from the digestion products of the enzyme trypsin on the protein. Trypsin was employed in the latter case to prevent the decomposition of tryptophane which is destroyed by heating with acids.

From a perusal of the above figures it can be readily seen that salmon protein contains the 6 amino acids studied in comparatively high percentages, with the exception of tyrosine which is present in low concentrations as compared to many other proteins. Although the amounts of the other 5 do not in all cases approach the maximum that have been found in other proteins, the distribution is such that salmon protein could serve as a source for these compounds, especially if a suitable procedure for obtaining all of the amino acids from the same hydrolysate could be employed.

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Local Anesthetic Action of Some Phthalic and Aminophthalic Acid Esters*

By L. W. Rowe†

A series of basic esters of phthalic and aminophthalic acids, prepared and reported upon chemically by Blicke and Otsuki (1, 2) and by Blicke and Castro (3), have recently been subjected to preliminary tests for local anesthetic action, as well as toxicity and irritant action. It was reported in these chemical publications that many of the compounds had been found to possess local anesthetic action and that a separate report would be made later. The investigation was made along similar lines to that reported for the aminonaphthoic acid esters (4).

EXPERIMENTAL

The 2 pharmacological methods used chiefly for determining local anesthetic efficiency were the rabbit cornea and the frog sensory nerve as proposed and described by Sollmann (5, 6).

Toxicity was determined by intraperitoneal injection into white mice, and only an approximate M. L. D. rather than an LD_{50} was obtained. Irritant action was largely determined from the effect of various concentrations upon the rabbit's eye.

In Table I are given the chemical names and empirical formulas of the series of compounds investigated in comparison with cocaine and procaine. These are all esters of phthalic or aminophthalic acid and will be given symbols for identification in the other tables. Nearly all of them were tested as salts such as the hydrochloride or the hydrobromide and as such are nicely water-soluble. The *p*H of a 1% solution in distilled water is given for some of the compounds.

In Table II the relative local anesthetic effectiveness of the compounds by both the rabbit cornea and the frog sensory nerve methods is shown, as well as approximate M. L. D.'s obtained by intraperitoneal injection of white mice. Data obtained from tests of cocaine and procaine are submitted for comparison. In all cases only that part of all the effectiveness data obtained is presented in the table, which shows the positive and more negative limits of the compounds.

DISCUSSION

The esters which give the best results by both the rabbit cornea and the frog sensory nerve methods and in addition are not irritating to the rabbit's eye in their effective dilutions are those of 3-aminophthalic acid, namely, CO 107, 108, 110, 113, 114 and 123. Of these 6, CO 110 (the *n*-propyl derivative) and CO 114 (the *n*-amyl derivative) are the most active, and the latter is the most active of the entire series. In fact, CO 114 is much more active than cocaine and just about as toxic, while it is fully as active as naphthocaine 4A (4).

Several of these groups, namely, esters of phthalic acid (EC 101 and CO 106), esters of phthalic acid monamides (CO 101, CO 102 and EC 100), ester of 3-aminophthalic acid monamide (CO 105), ester of 3-nitrophthalic acid CO 112 are comparatively ineffective as local anesthetics, cause local irritation, and so are deserving of no further consideration.

The other 2 groups, namely, esters of 3-aminophthalic acid and esters of 4-aminophthalic acid, comprising 24 and 10 compounds, respectively, also have some very unpromising members, such as CO 111, 115, 119, 120, 124, 125 and EC 111, 115.

Among the esters of 4-aminophthalic acid, EC 109 (*sec*-butyl) is about the best, but it is much less active than CO 114 or naphthocaine 4A, is irritating to the rabbit's eye even in 0.25% and is the most toxic of the group of 10 compounds.

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* From the Research Laboratories of Parke, Davis and Company, Detroit, Mich.

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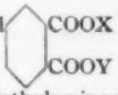
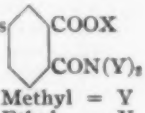
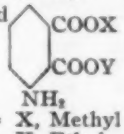
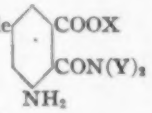
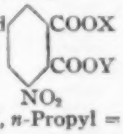
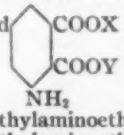
† The author acknowledges the assistance of Dr. H. C. Parke and of Mr. H. W. Pfeifle with certain phases of this work.

TABLE I.—NAMES, FORMULAS AND SYMBOLS OF THE ESTERS

	Formula	Symbol	pH of 1% Sol.
Esters of Phthalic Acid 			
1. Methyl = X, β-Diethylaminoethyl = Y, β-Diethylaminoethyl = X and Y	$C_{15}H_{21}O_4N.HCl$	EC 101	3.93
	$C_{20}H_{32}O_4N.HCl$	CO 106	4.72
Esters of Phthalic Acid Monamides 			
1. Methyl = X and Y	$C_{11}H_{13}O_3N$	CO 101	5.08
2. β-Diethylaminoethyl = X, Methyl = Y	$C_{16}H_{24}O_3N_2.HCl$	CO 102	5.10
3. β-Diethylaminoethyl = X, Ethyl = Y	$C_{18}H_{28}O_3N_2.HCl$	EC 100	4.87
Esters of 3-Aminophthalic Acid 			
1. β-Diethylaminoethyl = X, Methyl = Y	$C_{15}H_{23}O_4N_2.HCl$	CO 104	5.23
2. β-Diethylaminoethyl = X, Ethyl = Y	$C_{16}H_{24}O_4N_2.HCl$	CO 103	4.23
3. β-Diethylaminoethyl = X, Isopropyl = Y	$C_{17}H_{26}O_4N_2.HCl$	CO 109	4.57
4. β-Diethylaminoethyl = X, *n*-Propyl = Y	$C_{17}H_{26}O_4N_2.HCl$	CO 110	5.33
5. β-Diethylaminoethyl = X, Isobutyl = Y	$C_{18}H_{28}O_4N_2.HCl$	CO 108	4.63
6. β-Diethylaminoethyl = X, *sec*-Butyl = Y	$C_{18}H_{28}O_4N_2.HBr$	CO 117	5.76
7. β-Diethylaminoethyl = X, *n*-Butyl = Y	$C_{18}H_{28}O_4N_2.HCl$	CO 107	4.43
8. β-Diethylaminoethyl = X, *n*-Amyl = Y	$C_{19}H_{30}O_4N_2.HCl$	CO 114	4.37
9. β-Diethylaminoethyl = X, *n*-Hexyl = Y	$C_{20}H_{32}O_4N_2.HCl$	CO 118	3.79
10. β-Diethylaminoethyl = X, Lauryl = Y	$C_{28}H_{44}O_4N_2.Citrate$	CO 120	3.32
11. β-Diethylaminoethyl = X, Octadecyl = Y	$C_{22}H_{46}O_4N_2$	CO 119	4.85
12. *n*-Piperidinoethyl = X, *n*-Propyl = Y	$C_{16}H_{22}O_4N_2.HCl$	CO 113	5.51
13. γ-Di-*n*-butylaminopropyl = X, *n*-Propyl = Y	$C_{22}H_{36}O_4N_2.HCl$	CO 116	5.14
14. β,β-Dimethyl-γ-dimethylaminopropyl = X, *n*-Propyl = Y	$C_{18}H_{28}O_4N_2.HBr$	CO 123	6.3
15. Methyl = X, β-Diethylaminoethyl = Y	$C_{15}H_{22}O_4N_2.HCl$	CO 111	
16. Ethyl = X, β-Diethylaminoethyl = Y	$C_{16}H_{24}O_4N_2.HCl$	CO 115	4.90
17. Isopropyl = X, β-Diethylaminoethyl = Y	$C_{17}H_{26}O_4N_2.Citrate$	CO 125	3.63
18. *n*-Propyl = X, β-Diethylaminoethyl = Y	$C_{17}H_{26}O_4N_2.HBr$	CO 122	5.86
19. Isobutyl = X, β-Diethylaminoethyl = Y	$C_{18}H_{28}O_4N_2.HBr$	CO 121	5.72
20. *sec*-Butyl = X, β-Diethylaminoethyl = Y	$C_{18}H_{28}O_4N_2.Citrate$	CO 126	3.54
21. *n*-Butyl = X, β-Diethylaminoethyl = Y	$C_{18}H_{28}O_4N_2.HBr$	CO 127	5.28
22. *n*-Amyl = X, β-Diethylaminoethyl = Y	$C_{19}H_{30}O_4N_2.Citrate$	CO 128	3.58
23. *n*-Hexyl = X, β-Diethylaminoethyl = Y	$C_{20}H_{32}O_4N_2.Citrate$	CO 129	3.56
24. *n*-Propyl = X, β,β-Dimethyl-γ-dimethylaminopropyl = Y	$C_{18}H_{28}O_4N_2.HBr$	CO 124	3.97
Ester of 3-Aminophthalic Acid Monamide 			
β-Diethylaminoethyl = X, Ethyl = Y	$C_{18}H_{29}O_3N_3.HCl$	CO 105	4.78
Ester of 3-Nitrophthalic Acid 			
β-Diethylaminoethyl = X, *n*-Propyl = Y	$C_{17}H_{24}O_6N_2.HCl$	CO 112	4.12
Esters of 4-Aminophthalic Acid 			
1. Methyl = X, β-Diethylaminoethyl = Y	$C_{15}H_{23}O_4N_2.HCl$	EC 111	6.21
2. Ethyl = X, β-Diethylaminoethyl = Y	$C_{16}H_{24}O_4N_2.HCl$	EC 112	5.85
3. Isopropyl = X, β-Diethylaminoethyl = Y	$C_{17}H_{26}O_4N_2.Citrate$	EC 107	3.60
4. *n*-Propyl = X, β-Diethylaminoethyl = Y	$C_{17}H_{26}O_4N_2.HBr$	EC 104	5.41
5. Isobutyl = X, β-Diethylaminoethyl = Y	$C_{18}H_{28}O_4N_2.Citrate$	EC 108	3.57
6. *sec*-Butyl = X, β-Diethylaminoethyl = Y	$C_{18}H_{28}O_4N_2$	EC 109	2.45
7. *n*-Butyl = X, β-Diethylaminoethyl = Y	$C_{18}H_{28}O_4N_2.Citrate$	EC 110	3.59
8. Ethyl = X, β-Di-*n*-propylaminoethyl = Y	$C_{18}H_{28}O_4N_2.HCl$	EC 113	
9. Ethyl = X, β-(*N*-Piperidyl)-ethyl = Y	$C_{17}H_{24}O_4N_2.HCl$	EC 114	
10. Ethyl = X, β-(*N*-Morpholino)-ethyl = Y	$C_{16}H_{22}O_5N_2.HCl$	EC 115	

Thus of the 41 esters tested in a preliminary way, only CO 110 and CO 114 are highly active and non-irritating. They are more active than cocaine or procaine and are deserving of further study.

TABLE II.—LOCAL ANESTHETIC EFFECTIVENESS AND COMPARATIVE TOXICITY

No.	Product	M. L. D. Mg./Gm.	Frog Sensory Nerve		Rabbit Cornea	
1	EC 101	0.90	2.0%	5 min.	0.5%	Very slight
			1.0%	8 min.	1.0%	5 min. (irrit.)
2	CO 106	0.70	5.0%	12 min.	5.0%	Slight
3	CO 101	2.00	4.0%	4 min.	2.0%	None
			3.0%	12 min.	5.0%	4 min.
4	CO 102	0.18	5.0%	>15 min.	5.0%	None (irrit.)
5	EC 100	0.35	2.0%	>15 min.	2.0%	None
6	CO 104	0.50	2.0%	4 min.	2.0%	10 min.
			0.5%	12 min.	1.0%	5 min.
7	CO 103	0.30	0.5%	3 min.	1.0%	20 min.
			0.1%	11 min.	0.2%	5 min.
8	CO 109	0.15	0.1%	6 min.	1.0%	20 min.
			0.05%	10 min.	0.25%	7 min.
9	CO 110	0.25	0.10%	4 min.	0.25%	22 min.
			0.02%	14 min.	0.05%	3 min.
10	CO 108	0.15	0.25%	2 min.	0.25%	15 min.
			0.05%	9 min.	0.075%	4 min.
11	CO 117	0.20	0.20%	4.5 min.	0.5%	10 min. (irrit.)
			0.05%	12 min.	0.2%	Slight
12	CO 107	0.15	0.5%	2 min.	0.5%	40 min.
			0.05%	9 min.	0.1%	8 min.
13	CO 114	0.10	0.5%	3 min.	0.2%	40 min.
			0.05%	7 min.	0.05%	10 min.
					0.025%	6 min.
14	CO 118	0.10	2.0%	6 min.	0.2%	30 min.
			0.2%	8 min.	0.05%	5 min.
15	CO 120	0.15	2.0%	1.7 min.	2.0%	10 min. (very irrit.)
			0.5%	20 min.	1.0%	Some (irrit.)
16	CO 119	0.25	0.25%	4 min.	0.25%	None (irrit.)
			0.10%	15 min.		
17	CO 113	0.12	0.2%	3.2 min.	0.5%	30 min.
			0.025%	11 min.	0.1%	5 min.
18	CO 116	0.15	1.0%	4 min.	0.10%	30 min.
			0.5%	7 min.	0.025%	5 min.
19	CO 123	0.10	0.25%	33 min.	0.5%	35 min.
			0.05%	8.3 min.	0.1%	4 min.
20	CO 111	0.20	0.5%	3.5 min.	4.0%	7 min. (irrit.)
			0.10%	9 min.	1.0%	Never good
21	CO 115	0.15	0.5%	5 min.	1.0%	8 min. (irrit.)
			0.1%	9 min.	0.5%	Some
22	CO 125	0.20	2.0%	9 min.	1.0%	Fair (irrit.)
			1.0%	13 min.	0.5%	Slight (irrit.)
23	CO 122	0.12	0.5%	4 min.	1.0%	19 min. (irrit.)
			0.1%	8.3 min.	0.25%	8 min. (irrit.)
24	CO 121	0.12	0.5%	3 min.	1.0%	21 min. (irrit.)
			0.05%	8 min.	0.25%	6 min. (irrit.)
25	CO 126	0.18	2.0%	7 min.	1.0%	11 min. (irrit.)
			1.0%	12 min.	0.25%	3 min. (irrit.)
26	CO 127	0.12	2.0%	5 min.	0.5%	45 min. (irrit.)
			0.5%	7.3 min.	0.10%	10 min. (irrit.)
27	CO 128	0.15	1.0%	5 min.	0.5%	30 min. (irrit.)
			0.5%	12 min.	0.10%	10 min.
28	CO 129	0.18	2.0%	3.5 min.	0.25%	45 min. (irrit.)
			0.5%	8 min.	0.05%	5 min.
29	CO 124	0.20	2.0%	4 min.	1.0%	8 min.
			0.25%	8 min.	0.5%	Never good
30	CO 105	0.50	4.0%	>15 min.	5.0%	None
31	CO 112	0.60	2.0%	4 min.	1.0%	Some (very irrit.)
			0.5%	10 min.	0.5%	Slight (irrit.)
32	EC 111	0.45	1.0%	5 min.	2.0%	Some
			0.5%	10 min.	0.5%	Very slight
33	EC 112	0.25	0.5%	4 min.	1.0%	15 min.
			0.25%	8 min.	0.5%	10 min.
34	EC 107	0.15	2.0%	3 min.	0.5%	15 min.
			0.5%	8 min.	0.25%	3 min.
35	EC 104	0.12	0.25%	3.3 min.	0.5%	20 min.
			0.10%	9 min.	0.1%	3 min.
36	EC 108	0.15	2.0%	4.7 min.	0.5%	22 min.
			0.25%	9 min.	0.25%	10 min.

TABLE II.—LOCAL ANESTHETIC EFFECTIVENESS AND COMPARATIVE TOXICITY—(*Cont'd.*)

No.	Product	M. L. D. Mg./Gm.	Frog Sensory Nerve		Rabbit Cornea	
37	EC 109	0.10	1.0%	3 min.	0.25%	40 min. (irrit.)
			0.10%	7.5 min.	0.05%	Never good
38	EC 110	0.12	2.0%	5 min.	0.25%	30 min.
			0.5%	9 min.	0.10%	Fair
39	EC 113	0.30	0.5%	3.5 min.	1.0%	30 min. (irrit.)
			0.10%	13 min.	0.5%	7 min.
40	EC 114	0.20	0.5%	2.5 min.	1.0%	30 min. (irrit.)
			0.10%	10 min.	0.25%	9 min.
41	EC 115	0.80	4.0%	8 min.	4.0%	Never good
			2.0%	10 min.	2.0%	Slight
42	Cocaine	0.10	0.5%	2.5 min.	0.5%	16 min.
			0.10%	14 min.	0.10%	None
43	Procaine	0.40	1.0%	3 min.	2.0%	5 min.
			0.5%	11 min.	1.0%	None

SUMMARY

1. Forty-one esters of phthalic acid and of aminophthalic acid have been subjected to a preliminary examination for local anesthetic action, relative toxicity and irritant action.

2. The normal propyl ester and the normal amyl ester of 3-aminophthalic acid are highly active and satisfactory enough in other ways to be deserving of further study.

3. Most of the other esters are not particularly promising.

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The Preparation of Liquid Petrolatum Emulsions Using Gelatin and Domestic Gum*

By Charles Shackelford and Tulio Cordero†

Because of the scarcity of materials due to present world conditions, it has been necessary to develop mineral oil emulsions using gums and/or other emulsifying agents of strictly domestic origin.

The number of domestic substitutes which may be used to replace acacia, agar, tragacanth and other imported gums is rather limited. Work on the problem was confined to an investigation of the use of alginates and gelatin as the emulsifying agents.

The alginate used is a light brown powder easily soluble in hot or cold water. Chemically it is the sodium salt of alginic acid obtained by proper treatment from a kelp-like marine plant (*Mycrocystis pyrifera*) which grows off the West Coast of the United States.

The gelatins studied are of 2 types as follows:

1. A gelatin prepared from alkaline precursor, Bloom No. 150, isoelectric point at *p*H 4.7.

2. A gelatin prepared from an acid treated precursor, Bloom No. 225, isoelectric point at *p*H 8–9.

The requisites of a satisfactory liquid mineral oil emulsion for sale on a national scale are:

* Received October 6, 1942, from the Department of Research and Control, United Drug Company, Boston, Mass.

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1. It must be homogeneous and attractive in appearance.

2. It must be relatively stable for long periods of time to widely varying conditions of temperature.

2. It must be pleasant tasting.

4. It must be of the proper viscosity and must maintain this viscosity throughout its shelf life.

5. It must be nonfermentable.

6. It must lend itself to large-scale commercial production.

EXPERIMENTAL

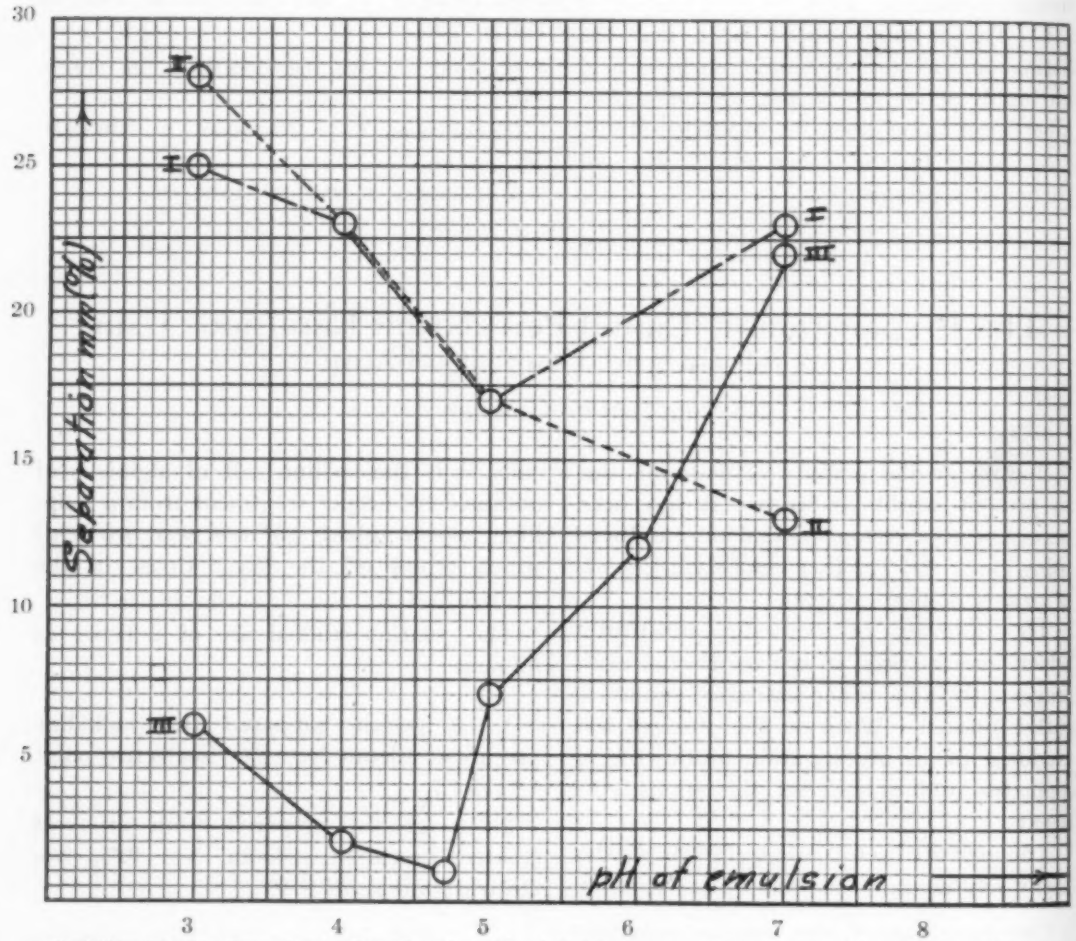
Procedure.—Essentially, the formula used consisted of heavy mineral oil 50% by volume, water 50% by volume, with added preservative (sodium benzoate) and flavoring materials.

For the experimental work, the oil was added to the solution of the gums and thoroughly mixed to form a primary emulsion. This emulsion was then passed through a hand homogenizer 3 times. Accelerated stability tests were made by keeping the samples at 100° F. for 30 days.

Gelatin Emulsions.—The first samples were made using gelatin as the sole emulsifying agent. The *p*H's of these samples were adjusted by using HCl and NaOH. Both gelatins, No. 1 and No. 2, exhibited less separation as they approached their isoelectric points. The concentration of the gelatin was 0.5%, *w/v*, based on the total volume of the emulsion.

These results are graphically illustrated by graph Nos. I and II. Our observations closely check those of Zhukov and Bushmakin (6) who noted maximum

RELATIONSHIP BETWEEN STABILITY AND *p*H OF MINERAL OIL EMULSIONS STABILIZED WITH GELATIN, GELATIN-SODIUM ALGINATE



Stability Testing Procedure: Samples placed in airtight glass jars (6 cm. x 12 cm.) to height of 10 cm.—kept in oven at 100° F. for 30 days. At end of 30-day period column of separated liquid at botton of jar is measured in mm. (%) and plotted against *p*H as above.

I, Gelatin No. 1; II, Gelatin No. 2; III, Gelatin No. 3, Sodium Alginate.

stability of benzine emulsions stabilized with gelatin at its isoelectric point. We were not able to confirm the results obtained by Tice (3, 4, 5). He states that mineral oil emulsions made with gelatin become more stable the farther the *p*H of the emulsion is removed from the isoelectric point of the gelatin used.

Effect of pH.—An important consideration is the influence of *p*H on taste and stability to bacterial decomposition. Our experience shows that the ideal *p*H for pharmaceutical oil emulsions is between 4 and 5. Therefore, gelatin No. 1 was chosen because of its maximum stabilizing action at its isoelectric point which coincides with the ideal *p*H for the emulsion.

Gelatin-Sodium Alginate Emulsion.—However, even though gelatin No. 1 shows optimum stabilizing action at *p*H 4–5, the emulsion so made was not considered sufficiently stable for large-scale production and sales. Therefore, an auxiliary emulsifying agent was used to increase viscosity, without causing gelation. This was done by incorporating 0.5% of sodium alginate in the emulsion.

The effect of *p*H on the separation of gelatin No. 1 and sodium alginate emulsion was studied and the results are shown by graph No. III. It will be noted that again minimum separation occurs at *p*H 4–5.

However, note that the separation is much less than in the sample made using gelatin No. 1 alone.

CONCLUSIONS

1. Gelatin is most efficient as an emulsifying agent for heavy mineral oil emulsions when the *p*H of the emulsion is at the isoelectric point of the gelatin.

2. Mineral oil emulsions made with gelatin require an auxiliary emulsifying agent to increase viscosity without gelation and thus increase stability.

3. A satisfactory and stable emulsion of mineral oil can be prepared by using gelatin with isoelectric point at *p*H 4–5 and sodium alginate.

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A Study of the Precipitation in Fluidextract of Senna*

By Virgil D. Wiebelhaus† and C. O. Lee‡

Arabian physicians of the ninth century are given credit for introducing senna to the medical profession (1). Senna leaves have been official in every issue of the U. S. Pharmacopœia. The fluidextract has been official since 1850.

The average annual importation of senna for the years 1929 to 1939, inclusive, was more than 2,000,000 lbs. (2). This is evidence of its importance and use.

In 1821 Lassaigne and Fenneulle (3) isolated a material from senna which they called cathartin. They felt that it was the active principle of the drug. Since then senna has been the object of much phytochemical research.

Fluidextract of Senna.—Fluidextract of senna is an unstable preparation. It continues to throw down a precipitate over a long period of time, becoming muddy in appearance and coating the sides of the container. Geiger (4) demonstrated that the official fluidextract was only about one-fourth as active as the crude drug and that the precipitate which it contained was as active as the liquid portion. The fact that 1 ml. of the fluidextract does not represent the active principles of 1 Gm. of the crude drug suggests an interesting problem.

Kaufman and Lee (5) showed that the addition of 1.5% to 2.5% of sodium borate to the fluidextract of senna reduced the amount of precipitate formed upon standing to a negligible amount, even for long periods of time. Since other basic substances, such as weak hydroxide solutions, did not reduce the formation of the precipitate in

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the fluidextract it was assumed that the borate radical was specific in its action. It is now suggested that the effect of sodium borate may be due to the formation of soluble anthranol-borate esters which resist oxidation to the corresponding and generally less soluble quinone derivatives. This is analogous to the mechanism of the Bohn-Schmidt reaction in which boric acid is employed to moderate the oxidation of various anthracene derivatives (6).

The ingestion of even small amounts of borates over prolonged periods of time manifests itself in undesirable physiological responses. For this reason we have been prompted to seek more acceptable ways of preventing deterioration in fluidextract of senna. The results of our study are being reported, in part, in this paper.

EXPERIMENTAL

I. Heat Treatment.—Three 240-Gm. samples of coarsely powdered senna were thoroughly moistened with distilled water and placed in the autoclave and subjected to a temperature of 123–126° C. at 19 lbs. pressure for 15 min., 30 min. and 45 min., respectively. Following treatment these samples were air-dried and then percolated in the official manner. Three control samples were percolated at the same time, according to the official procedure. The 6 fluidextracts were observed each 30 days for 270 days. The amounts of the precipitates which formed were measured in centimeters. Samples 1, 3 and 5 were prepared from the drug which had been subjected to the heat of the autoclave. Samples 2, 4 and 6 were the control samples, untreated in any manner. The results are tabulated in Table I.

The 15-min. period of treatment was not so effective in preventing the formation of a precipitate as the longer 30- and 45-min. periods. Later observations have led us to suggest that fluidextract of senna made from the heat-treated drug should be allowed to stand about 30 days before being filtered. This treatment yielded clear products, whereas those which were filtered immediately after completion usually showed slight precipitates.

II. Bioassay of Senna Preparations.—Since the official fluidextract is very unstable, a number of senna preparations were assayed as follows.

Twenty-one white rats were selected, weighed and placed in individual cages. Ten were males and 11 were females. Their weights varied from 180 to 260 Gm. They were divided into 3 groups of 7, and were fed a prepared meal which constituted a balanced ration. One group was tested every third or fourth day. The group to be tested was deprived of their feed at 10 a. m. At 4 p. m. they received test doses thoroughly incorporated with about 3 to 5 Gm. of feed. They were given water at all times. The cages had false bottoms. Below the false bottoms were trays containing paper toweling upon which the feces fell. Readings were made the following day at 10 a. m. Catharsis was regarded as positive if the stools, instead of being solid and discrete, were moist, flattened or adherent to the toweling. The tests were conducted during the summer months when the temperature was quite consistently high. The Minimal Cathartic Dose was taken as the minimum amount of test material required to produce catharsis in at least 5 of each 7 animals receiving the material.

As compared to control animals, the rats used in these tests made their normal weight gains. The following materials were tested:

1. Powdered senna.
2. Official fluidextract—freshly prepared.
3. Official Fluidextract—120 days old.
4. Freshly prepared fluidextract made from drug autoclaved 15 min.
5. Freshly prepared fluidextract made from drug autoclaved 30 min.
6. Freshly prepared fluidextract made from drug autoclaved 45 min.
7. Fluidextract made from drug autoclaved 30 min.; 120 days old when tested.

TABLE I

Age of Sample in Days	No. of Sample 1	2	3	4	5	6
0	Filtered	Filtered	Filtered	Filtered	Filtered	Filtered
30	0.1 cm.	1.3 cm.	VS	0.4 cm.	VS	1.0 cm.
60	0.2	1.4	VS	0.5	VS	1.2
90	Filtered	Filtered	VS	0.6	VS	1.3
180	VS[a]	0.8	VS	0.6	VS	1.4
210	VS	1.0	VS	0.8	VS	1.5
240	VS	1.2	VS	0.9	VS	1.7
270	VS	1.4	VS	1.2	VS	1.9

[a] VS = a very slight precipitate.

All samples were filtered immediately upon being prepared. Samples 1 and 2 were also filtered after 90 days. Sample 1 remained practically clear while the official Sample 2 continued to throw down a precipitate.

8. Freshly prepared "fluidextract" made by extracting with boiling water.
9. Freshly prepared "fluidextract" which had been heated to drive off the alcohol. It was made to volume with distilled water.
10. Fluidextract made from drug heated with alcohol at 62° C. for 5 hrs.
11. Fluidextract which had been heated at 62° C. for 14 days and filtered.
12. Fluidextract to which 0.6 Gm. of emulsin had been added per 120 ml. It was incubated at 37° C. for 3 weeks and filtered.
13. Fluidextract made from drug previously treated with emulsin in distilled water.
14. Fluidextract to which 2.5% of sodium borate had been added.
15. Fluidextract (120 ml.) to which 5 ml. of stronger ammonia had been added. After 1 week it had been filtered.
16. Senna marc.
17. Senna mucilage which had been precipitated from a fresh infusion by means of alcohol.
18. Dried and powdered precipitate from fluidextracts 30 to 120 days old.

The results are given in Table II. Powdered senna was taken as the basis for calculating the per cent of effectiveness, since the fluidextract, to satisfy Pharmacopœial requirements, should be fully as active as the drug from which it is made.

SUMMARY AND CONCLUSIONS

1. Sodium borate markedly reduced the precipitate which occurred in fluidextract of senna. This was probably due to the formation of soluble anthranol-borate esters which are resistant to oxidation.

2. All heat treatments reduced the amount of precipitate. Autoclaving the moistened drug for 30 to 45 min. at 123–126° C. under 19 lbs. pressure proved to be satisfactory. Preparations made from senna treated in this manner precipitated only very slightly if filtered after 30 days. They were as active as the official fluidextract and maintained their activity at least 120 days. It appears likely that their activity would remain at this level indefinitely since the precipitate which occurred after 30 days was negligible.

3. The freshly prepared official fluidextract of senna was only 30.7% effective and continued to lose strength rapidly The precipitate was active.

TABLE II

Sample No.[a]	Age of Sample in Days	Minimal Cathartic Dose	Effectiveness, %
1	...	2.0[c] grains	100.0
2	1	6.5 minims	30.7
3	120	19.5 minims	10.3
4	1	6.5 minims	30.7
5	2	6.5– 7.3 minims	30.7–27.4
6	3	6.5 minims	30.7
7	120	6.5– 7.3 minims	30.7–27.4
8	2	6.5 minims	30.7
9	4	6.5 minims	30.7
10	45	7.3 minims	27.4
11	70	16.2–17.9 minims	12.3–11.3
12	70	21.0 minims	9.5
13	60	19.5 minims	10.3
14	110	19.5 minims	10.3
15	52	14.6 minims	13.7
16	....	20.0 grains	10.0
17	1	77.6 grains[b]	[b]
18	30–120	6.2– 7.7 grains	32.2–25.9

[a] See above descriptions for details of each sample.
[b] Doubtful effects in 2 animals of 7.
[c] Work subsequent to this study would indicate that the minimum cathartic dose of senna for rats weighing about 200 Gm. is less than 2 grains. Results of our further study will be published later.

None of the preparations are more than 30.7% effective. The official fluidextract was 30.7% effective but lost strength rapidly upon aging. The amount of precipitate contained in such a preparation aged 120 days contained as much activity as the solution itself.

Fluidextracts made from autoclaved drug were just as effective as the official preparation and maintained this level of activity for at least 120 days. Whether the drug is autoclaved 15, 30 or 45 min. appears to have little effect on the activity. The borate-treated fluidextract was only 10.3% effective. The various heat treatments produced comparatively active and stable preparations.

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